Mutagenic Evaluation of Compound FDA 71-72 Glucono-Delta-Lactone 10/9/74

MUTAGENIC EVALUATION OF COMPOUND FDA 71-72 GLUCONO-DELTA-LACTONE

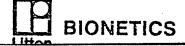
# ""SUBMITTED TO

FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

SUBMITTED BY

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# EVALUATION SUMMARY

Compound FDA 71-72, glucono-delta-lactone, was not genetically active, either directly or in the presence of organ homogenates, in any of the  $\underline{in\ vit^{vo}}$  assays employed in this evaluation.

DATE: October 1, 1974

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Mutagenic Evaluation of Compound FDA 71-72

### I. OBJECTIVE

The objective of this study was to assess the genetic activity of the metatorial in microbial assays with and without the addition of mammalian metabolic enzyme preparations.

#### II. MATERIALS

## A. Test Material

Glucono-delta-lactone

Pfizer #70123

## B. <u>Tissue Homogenates and Supernatants</u>

The tissue homogenates and 9,000 x g supernatants were prepared from liver, lung and testes of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and primate - Macaca mulatta adult males.

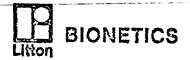
## C. <u>Indicator Organisms</u>

The indicator organisms used for all tests are described below:

- Saccharomyces cerevisiae, strain D4:  $\alpha$  ade 2-2 try 5-12 a, ade 2-1, try 5-27
- <u>Salmonella typhimurium</u>, strains:

# D. Reaction Mixture

The following reaction mixture was employed in the activation tests:



•	Component	Final Concentration/nl
1. 2. 3. 4.	TPN (sodium salt) Isocitric acid Tris buffer, pH 7.4 MgCl2 Isocitric dehydrogenase	6 րM 49 րM 28 րM 1.7 րM
6.	Tissue homogenate or cell fraction	6.3 Units

Components 1-4 were combined and frozen as a "core" reaction mixture to which the other components were added just prior to use.

# E. <u>Positive Control Compounds</u>

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

ASSAY	<u>CHEMICAL</u> <sup>a</sup>	SOLVENT	PROBABLE MUTAGENIC SPECIFICITY t
Non-activation	Ethylmethane sulfonate 2-Nitrosofluorene	Water or saline Dimethylsulfoxide <sup>C</sup>	BPS FS
	Quinacrine or Quinacrine mustard	Water or saline	FS
Activation	<ul><li>Dimethylnitrosamine</li><li>2-Acetylaminofluorene</li></ul>	Water or saline Dimethylsulfoxide <sup>C</sup>	BPS FS

a Concentrations given in the Results Section.

# III. METHODS

# A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against strains TA-1537 and D4 over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival dose was determined from the survival curve and the 1/4 and 1/2 50% doses calculated.



b BPS = base-pair substitution; FS = frameshift.

C Previously shown to be non-mutagenic, see Appendix.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

### B. Plate Tests

Only three bacteria strains were tested in qualitative plate tests. In the non-activation procedure, approximately 109 cells of a log phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (Test, Positive Control and Solvent Control) was done in duplicate. The results were scored as + or -. Concentrations of the positive control compounds are listed in the Results Section.

## C. Suspension Tests

### 1. Non-activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1 x  $10^9$  cells/ml and 5 x  $10^7$  cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in 30 ml plastic tissue culture flasks. Cells plus appropriate volume(s) of the test chemical were added to the flasks to give, a final volume of 2 ml. Solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the flasks were set in ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium. Samples from a 10-1 dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

#### 2. Activation

Bacteria and yeast cells were grown and prepared as described in the non-activation tests except that the cell densities were increased approximately five-fold for working stock suspensions. Measured amounts of the test and



control chemicals plus 0.25 ml of the stock cell suspension were added to a 30 ml plastic tissue homogenate. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non-activation tests.

# D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

#### 1. Mice

Male mice (sufficient to provide the necessary quantities of organs for testes, lung and liver homogenates) were killed by cranial blow, decapitated and bled. The three organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

#### 2. Rats

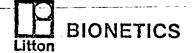
The same procedures as described for mice was used for this mammal.

#### 3. Primates

The liver, lungs and testes were aseptically removed from freshly killed adult male rhesus (M. mulatta) monkeys. Each organ was cut into a number of pieces each sufficient for one week's studies. The tissues were labeled and frozen at -80°C until needed. Tissue homogenates and 9,000 x g supernatants were prepared as described for mice.

# E. <u>Data Recording and Reporting</u>

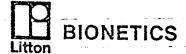
Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set was recorded, in ink, in bound data books. Information necessary for identification of the specific experiment as well as the presence of any contaminant microorganisms was recorded with each set of plate counts. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated.



Frequencies were mechanically calculated and double checked. All data presented in the Results Section of this report consists of the actual sum of all raw data plate counts and only the frequencies are calculated figures.

## IV. SOLUBILITY PROPERTIES OF THE TEST COMPOUND

- 1. NAME OR DESCRIPTION OF TEST COMPOUND: FDA 71-72
  GLUCONO-DELTA-LACTONE
- 2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY
  OF THE TEST CHEMICAL UNDERTREATMENT
  CONDITIONS: 0.067 M phosphate buffer, pH 7.4, was used as
  the solvent for this compound, and the compound was soluble
  under treatment conditions in all tests.
- 3. OTHER COMMENTS:



			<u> </u>		
		D4	TA-1537		
	Dose No.	% Concentration	% Concentration		
Range of concentrations of	1	1	1		
the test compound used to	2	2	2		
determine the 50% survival	3	3	• 3		
level	· 4	4	4		
	5	5	5		
	Dose No.	% Survival	% Survival		
Survival Results	→ Control	100	100		
	≟ 1	96	51		
Test Date: 7-8-74	2	94	30		
	3	88	18		
<b>***</b>	4	73	16		
	5	48	13		
	Dose	% Concentration	% Concentration		
Concentrations of the test	Plate Test		0.50%		
chemical required for	- 1/4 50% Survival	1.25%	0.25%		
mutagenicity tests	½ 50% Survival	2.5 %	0.50%		

#### VI. NON-ACTIVATION PLATE TESTS

DATE: 8-30-74 TA-1535 TA-1537 TA-1538 Test Compound Concentration/plate T-1 T-2 T-1 T-2 T-1 T-2 0.05 ml undi-EMS PC 1 luted chemical QM 0.25 mg NF 0.25 mg SC SALINE DMSO <10%

NOTE: = positive control

= solvent control

= trial 1 = trial 2

= ethyl methanesulfonate

= quinacrine mustard = nitrosofluorene

DMSO = dimethyl sulfoxide

= contamination present

**.			DATE: 8-30-74							
	•		TA-1535	TA-1537	TA-1538					
Test	Compound <b>→</b>	Concentration	T-1 T-2	T-1 T-2	• T-1 T-2					
TC	FDA 71-72	0.50%								

TC = test compound T-1 = trial 1 T-2 = trial 2 NOTE:

SPECIES:	MOUSE				•	DATE	: 8-30	)-74	<u> </u>
				TA-1	535	TA-1	537	TA-1	538
Test	Organ	Compound	Concentration/plate	T-1	T-2	T-1	T-2	T-1	7-2
PC	Li	DMNA	25 umoles	+	+			•	
	. *	AAF <sup>£</sup>	1.25 mg			+	+	+	+
	Lu	DMNA	25 μmoles	+/-	+/-				A. and the second
		AAF <sub></sub>	₫ 1.25 mg					-	-
	T	DMNA	25 μmoles		-				و ا نه بازندست تصمیم
; ;		AAF z	1.25 mg			-	_	_	-
SC		DMNA	25 μmoles	-	<u>-</u> .				
		AAF -	1.25 mg			-	_		400
	-	Saline	-	3 -					
	-	DMSO	<10%				-	<u> </u>	-

NOTE:

PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine

= liver

= luna

= testes

T-1 = trial 1

T-2 = trial 2 DMSO = dimethyl sulfoxide

(c) = contamination present



SPECIES	S: MOUSE	DATE: 8-30-74								
	ž., .			_TA-1	TA-1535		TA-1537		_TA-1538	
Test	Organ	Compound	Concentration	T-1	T-2	•	T-1	T-2	• T-	1 T-2
тс	Li	FDA 71-72	0.50%	_	-		_	-	-	.   _
•	Lu	FDA 71-72	0.50%	-	1		-	-	-	<b>-</b>
	τ	FDA 71-72	0.50%	_	-		-	-	_	

NOTE: TC = test compound<sup>2</sup>
Li = liver
Lu = lung
T = testes
T-1 = trial 1
T-2 = trial 2

SPECIES	: RAT					DATE	: 8-	30-74	
				TA-1	535	TA-	537	TA-	1538
Test	Organ	Compound	Concentration/plate	T-1	T-2	T-1	T-2	T-1	T-2
PC	Li	DMNA	25 μmoles	+	+			•	
·		AAF	1.25 mg			. +	+	+	+
	Lu	DMNA	25 µmoles						
•		AAF ,	1.25 mg		4		-		_
	T	- DMNA	25 μmoles	-					
•		AAF Ž	1.25 mg			-		-	
sc	-	DMNA	25 umoles	-	-				
•		AAF T	1.25 mg					_	-
		Saline		*	-	in.			
) 1		DMSO	<10%			_	-		

NOTE:

PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver

= testes

T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

¦ Li

SPECIES	S: RAT			DATE: 8-30-74						
			TA-1535		TA-1	537	TA-1538			
Test	Organ	Compound	Concentration	T-1	T-2	T-1	T-2	•T-1	T-2	
TC	Li	FDA 71-72	0.50%	-	_	_			_	
•	Lu	FDA 71-72	0.50%	_	-	_	-		_	
	T	FDA 71-72	0.50%		_	_	_			

NOTE: TC = test compound

Li = liver

Lu = lung

T = testes

T-1 = trial 1

T-2 = trial 2

SPECIES:	MONKEY					DATE	: 8-30	D-74	
				_TA-1	535	TA-1	537	TA-1	538
Test	Organ	Compound	Concentration/plate	T-1	T-2	T-1	T-2	T-1	T-2
PC	Li	DMNA	25 µmoles	+	+				
	<b>*</b>	AAF	ધ 1.25 mg			+	+	±	+
	Lu	DMNA	25 µmoles	+/-	+/-				
•		AAF	₫ 1.25 mg			_	-	-	-
	Т	DMNA	25 μmoles	_	-				
		AAF	: 1.25 mg			-	-	-	-
SC		DMNA	25 µmoles	-	-	*****			
÷	•	AAF	1.25 mg			-	_	_	-
**	40	Saline	•	4.4					,
	*	DMSO	<10%			-	-	-	-

NOTE:

PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver

= lung

= testes

T-1 = trial ]

T-2 = trial 2 DMSO = dimethyl sulfoxide (c) = contamination present



SPECIES	S: MONKE	Y					DATE	: 8-3	0-74	
					535	· •	TA-1537		TA-1538	
Test	Organ	Compound	Concentration	T-1	T-2		T-1	T-2	• T-	1 T-2
TC	Li	FDA 71-72	0.50%	_	_		-		-	
•	Lu	FDA 71-72	0.50%	_	-		_	-	-	
	T	FDA 71-72	0.50%	_	-		-	-	-	_

NOTE: TC = test compound Li = liver Lu = lung T = testes T-1 = trial 1 T-2 = trial 2

#### NON-ACTIVATION SUSPENSION TESTS VIII. : STRAINS SETATION ALL STRAINS : POSITIVE AND SOLVENT CONTROL RESULTS

DATE: 8-16-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	TA-1535	EMS	0.05 %	10.31	2,628 .	254.90
	TA-1537	QM	0.01 mg/ml	2.79	59	21.15
	TA-1538	NF	1.25 mg/ml	2.66	43**	16.17
sc	TA-1535	SALINE	_	8.75	9	1.03
	TA-1537	SALINE		3.70	12	3.24
,	TA-1538	DMSO	4-	5.17	9	1.74

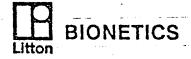
NOTE: PC = positive control

SC = solvent control

EMS = ethyl methanesulfonate

QM = quinacrine mustard

NF = nitrosofluorene DMSO = dimthyl sulfoxide



DATE: 8-16-74

Test	Indicator Strain	Compound	Concentration	Total <u>his+</u> Cells/ Revertants/ mlx10 <sup>8</sup> ml	his+ Revertants/10 <sup>8</sup> Survivors
тс-н	TA-1535	FDA 71-72	0.50%	7.82 (89) 10	1.28
TC-L	TA-1535	FDA 71-72	0.25%	5.22 (60) 9	1.72
	`				
тс-н	TA-1537	FDA 71-72	0.50%	3.04 (82) 5	1.64
TC-L	TA-1537	FDA 71-72	0.25%	4.16 (112) 11	2.64
				•	
С-Н	TA-1538	FDA 71-72	0.50%	4.64(90) 6	1.29
TC-L	TA-1538	FDA 71-72	0.25%	4.13 (80) 12	2.91
. •				•	

TC-H = test compound high dose
TC-L = test compound low dose
( ) = percent survival NOTE:



# WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

rgan Li Lu T -	Compound  DMNA  DMNA  DMNA  DMNA  DMNA  SALINE 5-74  Compound  AAF  AAF  AAF  AAF  DMSO 6-74	Concentration  100 µmoles/ml  100 µmoles/ml  100 µmoles/ml  100 µmoles/ml  Concentration  1.25 mg/ml  1.25 mg/ml  1.25 mg/ml  1.25 mg/ml	Total Cells/mlx108 5.71 4.99 7.25 10.55 5.80  Total Cells/mlx108 2.67 1.85 2.90 2.11 1.83	Strain TA-15  his+ Revertants/ ml  2,233  49  5  11  11  Strain TA-15:  his+ Revertants/ ml  45  11  16  6  11	his+ Revertants/10 <sup>8</sup> Survivors  391.07  9.82  0.69  1.04
Lu T - 7-2	DMNA DMNA DMNA SALINE 5-74  Compound AAF AAF AAF AAF DMSO	100 pmoles/ml 100 pmoles/ml 100 pmoles/ml 100 pmoles/ml  Concentration 1.25 mg/ml 1.25 mg/ml	4.99 7.25 10.55 5.80 Total Cells/mlx10 <sup>8</sup> 2.67 1.85 2.90 2.11	49 5 11 11 Strain TA-15: his+ Revertants/ ml 45 11 16 6	9.82 0.69 1.04 1.90 37 Mis+ Revertants/10 <sup>8</sup> Survivors 16.85 5.95 5.52 2.84
T	DIANA DANA SALINE 5-74  Compound AAF AAF AAF AAF DMSO	100 pmoles/ml 100 pmoles/ml  Concentration 1.25 mg/ml 1.25 mg/ml	7.25 10.55 5.80 Total Cells/ mlx10 <sup>8</sup> 2.67 1.85 2.90 2.11	5 11 11 Strain TA-15: his+ Revertants/ ml . 45 11 16 6	0.69 1.04 1.90 37 his+ Revertants/10 <sup>8</sup> Survivors 16.85 5.95 5.52 2.84
rgan Li Lu T -	DMNA SALINE 5-74  Compound AAF AAF AAF AAF DMSO	Concentration 1.25 mg/ml 1.25 mg/ml 1.25 mg/ml	10.55 5.80 Total Cells/ mlx10 <sup>8</sup> 2.67 1.85 2.90 2.11	11 Strain TA-15:  his+ Revertants/ ml . 45 11 16 6	1.04 1.90 37 his+ Revertants/10 <sup>8</sup> Survivors 16.85 5.95 5.52 2.84
rgan Li Lu T -	SALINE 5-74  Compound  AAF  AAF  AAF  AAF  AAF  DMSO	Concentration 1.25 mg/ml 1.25 mg/ml 1.25 mg/ml	5.80  Total Cells/mlx108  2.67  1.85  2.90  2.11	Strain TA-15:  his+ Revertants/ ml  45  11  16  6	1.90 37  his+ Revertants/10 <sup>8</sup> Survivors 16.85 5.95 5.52 2.84
rgan Li Lu T -	Compound  AAF  AAF  AAF  AAF  AAF  AAF  DMSO	1.25 mg/ml 1.25 mg/ml 1.25 mg/ml	Total Cells/mlx10 <sup>8</sup> 2.67 1.85 2.90 2.11	Strain TA-15:  his+ Revertants/ ml  45  11  16  6	37  his+ Revertants/10 <sup>8</sup> Survivors  16.85  5.95  5.52 2.84
rgan Li Lu T -	Compound  AAF  AAF  AAF  AAF  AAF  DMSO	1.25 mg/ml 1.25 mg/ml 1.25 mg/ml	Cells/mlx108 2.67 1.85 2.90 2.11	his+ Revertants/ ml . 45 . 11 . 16 . 6	his+ Revertants/10 <sup>8</sup> Survivors 16.85 5.95 5.52 2.84
Li Lu T	AAF AAF AAF AAF DMSO	1.25 mg/ml 1.25 mg/ml 1.25 mg/ml	Cells/mlx108 2.67 1.85 2.90 2.11	his+ Revertants/ ml . 45 . 11 . 16 . 6	his+ Revertants/10 <sup>8</sup> Survivors 16.85 5.95 5.52 2.84
Lu T	AAF AAF DMSO	1.25 mg/ml 1.25 mg/ml	1.85 2.90 2.11	11 16 6	5.95 5.52 2.84
T -	AAF AAF DMSO	1.25 mg/ml	2.90 2.11	16 6	5.52 2.84
-	AAF DMSO		2.11	6	2.84
- 7-26	DMS0	1.25 mg/ml			
7-26			1.83	11	6.01
7-26	6-74	•			0.01
				Strain TA-153	38
rgan	Compound	Concentration	Total Cells 6 mlx10	<u>his</u> + Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
Li	AAF	1.25 mg/ml	2.36	53	22.46
Lu	AAF	1.25 mg/ml	2.40	16	6.67
T	AAF		2.35	10	4.26
-	AAF	1.25 mg/ml	2.75	13	4.73
_	DMSO .	•	2.26 (	c) 6	2.65
C = : AF = : MNA = : i = : u = :	solvent and 2-acetylami dimethylnit liver lung testes	d chemical contro nofluorene crosamine	ls		tamination prese
i	T = = = = = = = = = = = = = = = = = = =	T AAF  - AAF  - DMSO  = positive column and a second	T AAF 1.25 mg/ml  - AAF 1.25 mg/ml  - DMSO -  = positive control = solvent and chemical control F = 2-acetylaminofluorene INA = dimethylnitrosamine = liver = lung	T AAF 1.25 mg/ml 2.35  - AAF 1.25 mg/ml 2.75  - DMSO - 2.26 (and the strength of the strength	T AAF 1.25 mg/ml 2.35 10  - AAF 1.25 mg/ml 2.75 13  - DMSO - 2.26 (c) 6  = positive control (c) = controls  = solvent and chemical controls  AF = 2-acetylaminofluorene  INA = dimethylnitrosamine     = liver     = lung     = testes

BIONETICS

# ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

SPECIE	S: Mous	SE				
DATE:	7-24-74			St	rain TA-15	535
Test	Organ	. Compound	Concentration	Total Cells/ Re mlx10 <sup>8</sup>	his+ vertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
· TC	Li	FDA 71-72	Н	6.55(113)	11	1.68
	•	· FDA 71-72	<u>L</u>	8.70(150)	23	2.64
	Lu	FDA 71-72	Н	2.73(47)	4	1.47
•		FDA 71-72	<u> </u>	6.30(109)		0.79
	<u>. T</u>	FDA 71-72	: Н	2.88(50)	2 .	0.69
· \		FDA 71-72	<u>L</u>	6.75(116)	10	1.48
DATE:	7-25	-75		St	rain TA-15	537
TC	Li	FDA 71-72	Н	3.26(178)	7	2.15
		FDA 71-72	L	4.69(256)	7	1.49
	Lu	FDA 71-72	н *	1.53(84)	9	5.88
\		FDA 71-72	L .	2.15(117)		0.93
	<u> </u>	FDA 71-72	Н	2.44(133)	6	2.46
•	·	FDA 71-72	L	2.95(161)	10	3.39
DATE:	7-26-	74		St	train TA-15	538
TC	Li	FDA 71-72	Н * 100	1.58(70)	7	4.43
•		FDA 71-72	~ L	·1':01(c)(4	5) 6	5.94
	Lu	FDA 71-72	Н	1.71(76)	6	3.51
		FDA 71-72	L	2.06(91)	9 ·	4.37
	Т	FDA 71-72	Н	2.03(90)	6	2.96
	•	FDA 71-72	L	1.57(69)	7	4.46

NOTES: H = high dose L = low dose TC = test compound

Li = liver Lu = lung T = testes

(c) = contamination present

( ) = percent survival



# WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

DATE:	8-	7-74	· · · · · · · · · · · · · · · · · · ·		Strain TA-15	35
Test	Organ	Compound	Concentration	Total Cells/ mlx108	his+ Revertants/ ml	<u>his</u> + Revertants/10 <sup>8</sup> Survivors
PC	Li	ÓMNA	100 pmoles/ml	6.54	4,269	652.75
	<u>Lu</u>	DMNA	100 umoles/ml	6.46	12	18.46
	<u> </u>	DMNA	100 µmoles/ml	9.37	3	0.32
SC		DMNA	100 µmoles/ml	5.74	11	1.92
	-	SALINE		4.17	8	1.92
DATE:	8-8	8-74			Strain TA-15:	37
Test	Organ	Compound	Concentration	Total Cells/ mlx108	his+ Revertants/**	<u>his</u> + Revertants/10 <sup>8</sup> Survivors
PC	<u>Li</u>	AAF	1.25 mg/ml	1.25	· 38	30.40
	Lu	AAF	1.25 mg/ml	6.50	33	5.08
<b></b>	T	AAF	1.25 mg/ml	4.53	18	3.97
SC	_	<b>A</b> AF	1.25 mg/ml	5.62	6	1.07
· · · · · · · · · · · · · · · · · · ·	**	DMS0	***	5.88	9	1.53
DATE:	8-9	9-74	•		Strain TA-153	38
Test	Organ	Compound	Concentration	Total Cells/ mlx108	<u>his</u> + Revertants/ ml	<u>his</u> + Revertants/10 <sup>8</sup> Survivors
PC	<u>Li</u>	- AAF	1.25 mg/ml	2.84	165	58.01
	Lu	AAF	1.25 mg/ml	3.95	20	5.06
· · · · · · · · · · · · · · · · · · ·	Ţ	AAF	1.25 mg/ml	5.09	26	5.11
SC ·		AAF .	1.25 mg/ml	3.88	10	2.58
	-	DMSO	***	4.21	8	1.90
NOTE:	SC = AAF = DMNA = Li = Lu = T	positive co solvent and 2-acetylamid dimethylnid liver lung testes dimethyl so	d chemical control inofluorene trosamine	s	(c) = contai	mination present

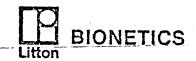
# ACTIVATION SUSPENSIO 1 TESTS ? WILDERTS RETADIONELLA INDICATE RETA

SPECIES	S: RAT		•					
DATE:	8-7-74	1		Stra	Strain TA-1535			
Test	<b>O</b> rgan	Compound	Concentration	Cells/ Reve	is+ rtants/ ml	his+ Revertants/10 <sup>8</sup> Survivors		
TC	<u>Li</u>	FDA 71-72	Н		14	4.70		
		- FDA 71-72	L	3.82 (92)	27	<b>7.</b> 07 ,		
	Lu	FDA 71-72	Н	. 0.67(16)	15	22.39		
-		FDA 71-72	<u>L</u>	3.50(84)	19	5.43		
	T	FDA 71-72	Н	1.80 (43)	14	7.78		
		FDA 71-72	<u>L</u> ·	3.72 (89)	14	3.76		
DATE:	8-8-74			Stra	in TA-15	37		
TC	Li	FDA 71-72	Н	6.26(106)	6	0.96		
		FDA 71-72	<u> </u>	5.65 (96)	18	3.19		
	Lu	FDA 71-72	H	1.74 (30)	8	4.50		
		FDA 71-72	L ·	3.99(68)	11	2.76		
	T	FDA 71-72	Н	3.00(51)	8	2.67		
		FDA 71-72	L	4.49(76)	11	2.45		
DATE:	8-9-7	4		Stra	in TA-15	38		
TC	Li	FDA 7,1-72	H *+0.	1.57(37)	17	10.83		
4		FDA 71-72	w L ×	.3.,61 (86)	<b>I</b> .1	3.05		
	Lu	FDA 71-72	Н	2.96(70)	6	2.03		
•		FDA 71-72	L	3.94 (94)	2 ·	0.51		
	T .	FDA 71-72	Н		11	4.78		
		FDA 71-72	\ L	4.79(114)	17	3.55		

NOTES: H = high dose L = low dose TC = test compound

Li = liver Lu = lung T = testes (c) = contamination present

) = percent survival



# ACTIVATION SUSPENSION TESTS: WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

SPEC	IES:	Rat:				
DATE	:	9-16-74	(Repeated Doses)		Strain TA-15	35
Test	Organ	Compound	• Concentration	Tctal Cells/ mlx108	his+	his+ Revertants/10 <sup>8</sup> Survivors
PC	<u>Li</u>	DMNA	100 μmoles/ml	4.19	3,155	752.98
	<u>Lu</u>	DMNA	100 μmoles/ml	2.92	7	2.40
	<u> </u>	DMNA	100 μmoles/ml	3.60	5	1.39
SC		DMNA	100 µmoles/ml	3.43	5	
		SALINE	_'	3.99	5	1.46
DATE:	<del></del>				Strain TA-153	1.25
Test	Organ	Compound	Concentration	Total Cells/ mlx108	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml			
	Lu	AAF	1.25 mg/ml			•
\	<u>T</u>	AAF	1.25 mg/ml			
SC		AAF	1.25 mg/ml			
<del></del>	-	DMSO		_		
DATE:					Strain TA-1538	2
		٠	1.11	Total	his+	
Test	Orgán	Compound	Concentration		Revertants/ m1	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml			
	Lu	AAF	1.25 mg/m1	•		
	T	AAF	1.25 mg/m7 ·			
SC	*	AAF	1.25 mg/ml			
·	<u> </u>	DMSO			**************************************	
NOTE:	SC = AAF = DMNA = Li = Lu = T =	positive co solvent and 2-acetylami dimethylnit liver lung testes dimethyl su	chemical controls nofluorene rosamine	•	(c) = contamina Project No	
ſ					ojece no	

BIONETICS

22a

# ACTIVATION SUSPENSION TESTS . WITH SALMONELLA INDICATOR STRAINS

SPECIE	ES: Ra	t					
DATE:	9-	16-74	(Repeated Doses)		Strain	TA-15	535
Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>			his+ Revertants/108 Survivors
TC	Li		Н		· · · · · · · · · · · · · · · · · · ·	<del></del>	
	·	FDA 71-7	<sup>72</sup> L	4.51	(113) 9		2.00
	Lu	FDA 71-7		. 2.50	(63) 8		3.20
	<u>T</u>	· FDA 71-7	L 2 H L	2.77	(69) <sub>6</sub>		2.17
DATE:		<del> </del>			Strain	TA-15:	37
TC	Li		Н				
	• • •		L				•
<b>\</b>	<u>. Lu</u>		H			<del></del>	
	T	· · · · · · · · · · · · · · · · · · ·	L H				
			L			<del></del>	
DATE:					Strain 7	A-153	18
TC ·	<u>Li</u>		H	. 124			<b></b>
•	Lu		<u>L</u> H		*		
	T		н.				
<del></del>			L				
NOTES:	L = low TC = test Li = liv Lu = lun T = tes	g tes		( ) = p	ercent su	ırviva	
\ \	(c)= con	tamination	present	•	•		

BIONETICS

# WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTRO - RESULTS

ATE:				· · · · · · · · · · · · · · · · · · ·	Strain TA-153	5
[est	Organ	Compound	; • Concentration	Total Cells/ mlx108	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	DMNA	100 umoles/ml			
	<u>Lu</u>	DMNA	100 µmoles/ml			
	T	DMNA	100 μmoles/ml			
SC	-	DMNA	100 µmoles/ml	*		
		SALINE				L.
DATE:					Strain TA-153	
Test	Organ	Compound	Concentration	Total Cells/ mlx108	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml			
	Lu	AAF	1.25 mg/ml			
	T	AAF	1.25 mg/ml		·	
SC	_	AAF	1.25 mg/ml			
-	_	DMS0	•			• .
DATE:	9-27		ated Doses)		Strain TA-15	38
Test	Organ	Compound	Concentration	Total Cells/ mlx108	his+ Revertants/ ml 4	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml	4.49	147	32.74
FU	Lu	AAF	1.25 mg/ml	2.99	40	13.38
	T	AAF	1.25 mg/ml	ì		
SC		AAF	1.25 mg/ml	5.07	40	7.89
<b>3</b> C		DMS0		4.72	40	8.47

AAF = 2-acetylaminofluorene DMNA = dimethylnitrosamine

= liver Li = lung Lu

T = testes DMSO = dimethyl sulfoxide



# ACTIVATION SUSPENSION TESTS WITH SALMONELLA INLICATOR STRAINS

DATE:				Strain TA-1535			
Test	Organ	Compound	Concentration	Total Cells/ mlxl0 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors	
TC	Li		Н				
10			·L				
	Lu		Н				
			; <u>L</u>		•		
	T		Н				
			<u> </u>		-		
DATE:					Strain JA-1	53/	
TC	Li		Н				
			L				
·	Lu		Н				
`			<u> </u>				
	T		H				
			<u> </u>		Chair TA	1520	
DATE:	9-27	-74 (Repeated	l Dose)	•	Strain TA-	1330	
TC	Li	FDA 71-72	<u>н</u>	2.30 (	49) 44	19.13,	
•			<u> </u>			>	
	<u>Lu</u>		<u>н</u>				
			L				
	<u>T</u>		Н		· · · · · · · · · · · · · · · · · · ·		
			L				
NOTES:	H = 1	nigh dose low dose		( )	) = percent	survival.	
	TC = 1	test compound					
		liver lung		•		ند. معاد المسكور أو العاد الدار	
	T = :	testes	•			:	
	(c) =	contamination	present	•	•	بور در این	
					Project No	002468	



BIONETICS

22d

# ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS: POSITIVE AND SOLVENT CONTROL RESULTS

PECIES	S: MON	KEY			C TA 3.50	
DATE:	9-5	-74			Strain TA-153	
Test	Organ	Compound	Concentration	Total Cells/ mlx108	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	DMNA	100 µmoles/ml	6.49	3,117	480.28
	Lu	DMNA	100 μmoles/ml	6.03	19	3.15
	T	DMNA	100 µmolès/ml	5.63	14	2.49
SC	-	DMNA	100 umoles/ml	6.40	23	· 3.59
	-	SALINE		5.11	10	1.96
DATE:	9	7-74			Strain TA-15	37
Test	Organ	Compound	Concentration	Total Cells/ mlx108	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	 Li	AAF	1.25 mg/ml	5.47	84	15.36
	Lu	AAF	1.25 mg/ml	5.64	17	. 3.01
		AAF	1.25 mg/ml	4.57	19	4.16
SC		AAF	1.25 mg/ml	5.67	40	7.05
		DMS0	•	6.20	44	7.10
DATE:	Q_	27-74	4 01		Strain TA-15	38
Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	Revertants/	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml	6.68	305	45.66
• •	Lu	AAF	1.25 mg/ml	5.09	52	10.22
-	T	AAF	1.25 mg/ml	5.15	33	6.41
SC		AAF +	1.25 mg/ml	5.07	40	7.89
30		DMSO		4.72	40	8.47

NOTE: PC

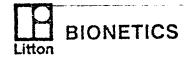
= positive control
= solvent and chemical controls SC

(c) = contamination present

AAF = 2-acetylaminofluorene DMNA = dimethylnitrosamine

= liver Li = lung Lu = testes T

DMSO = dimethyl sulfoxide



# ACTIVATION SUSPENSION TESTS WITH SALMONELLA INDICATOR STRAINS

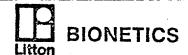
DATE:	9-5-	74		St	Strain TA-1535			
Test	Organ	Compound	Concentration	Total Cells/ Re mlx10 <sup>8</sup>	his+ vertants/ ml	his+ Revertants/10 <sup>6</sup> Survivors		
TC	Li	FDA-71-72	. Н	6.53 (128)	16	2.45		
		.FDA 71-72	·L	6.68 (131)	25	3.74		
	Lu	FDA 71-72	Н	4.16 (81)	11	2.64		
	1	FDA 71-72	Ĺ	5.40 (106)	12	2.22		
	T	FDA 71-72	Н	4.26 (83)	14	3.29		
		FDA 71-72	L	4.86 (95)	11	2,26		
DATE:	9-17-	74		St	rain TA-15	537		
TC	Li	FDA 71-72	Н	4.13 (67)	34	8.23		
		FDA 71-72	L	4.36 (70)	34	7.80		
`	Lu	FDA 71-72	Н	3.16 (51)	17	5.38		
		FDA 71-72	L	3.97 (64)	13	3.27		
	T	FDA 71-72	Н	1.90 (32)	15	7.89		
		FDA 71-72	L	3.83 (62)	88	2.09		
DATE:	9-27-	74	4 411	. \$1	rain TA-1	538		
TC	Li	FDA 71-72	₩ .	2.59 (55)	37	14.29		
		FDA 71-72	L	2.76 (58)	33	11.96		
	<u>Lu</u>	FDA 71-72	Н	2.67 (57)	25	9.36		
		FDA 71-72	L	6.44 (136)	65	10.09		
	Τ	FDA 71-72	Н	3.63 (77)	46	12.67		
		FDA, 71-72	L	3.91 (83)	36	9.21		
NOTES:		gh döse		) = percent	survival			

NOTES: H = high dose L = low dose

TC = test compound

lung testes

(c) = contamination present



# NON-ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4 Χ.

DATE: 7-29-74

		· .		Strai	n D4		
Test	Сотроила	Concentration	Total Population Screeneda	Numb Conve Ade <sup>+</sup>	er of rtantsb Try <sup>+</sup>	Converta 10 <sup>5</sup> Sur Ade <sup>+</sup>	nts Per vivors Try+
PC	EMS	1.0 %	4.71	479	315	101.70	66.88
SC	Saline	<b>-</b>	4.39	117	14	26.65	3.19

NOTE: PC = positive control
SC = solvent control
EMS = ethyl methanesulfonate
a = number x 10<sup>5</sup>
b = number at 10<sup>-1</sup> dilution

# NON-ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 7-29-74

			,	Strain D4	
Test	Compound	Concentration	Total Population Screened <sup>a</sup>	Number Convertantsb Ade+ Try+	Convertants Per 10 <sup>5</sup> Survivors Ade <sup>+</sup> Try <sup>†</sup>
TC	FDA 71-72	. H	4.30 (98)	79 13	18.37 3.02
	FDA 71-72	L ,	4.55 (104	90 11	19.78 2.42

NOTE: TC = test compound

H = high dose
L = low dose
a = number x 10<sup>5</sup>
b = number at 10<sup>-1</sup> dilution
( ) = percent survival



# ACTIVATION SUSPENSION TESTS . WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

IES: M	OUSE	· · · · · · · · · · · · · · · · · · ·		DATE:	7-29-	74	
				Strain	n D4		
Organ	Compound	Concentration	Total Population Screened <sup>a</sup>			Converta 10 <sup>5</sup> Sur Ade <sup>+</sup>	
Li	DMNA	150 µmoles/ml	2.90	237	67	81.72	23.10
Lu	DMNA	150 µmoles/ml	3.23	68	27	21.05	8.36
Т	DMNA	150 μmoles/ml	3.38	61	11	18.05	3.25
	DMNA	150 µmoles/ml	2.89	42	13	14.53	4.50
-	SALINE	-	2.75	89	6	32.36	2.18
	Organ Li Lu	Organ Compound Li DMNA Lu DMNA T DMNA - DMNA	Organ Compound Concentration  Li DMNA 150 µmoles/ml  Lu DMNA 150 µmoles/ml  T DMNA 150 µmoles/ml  - DMNA 150 µmoles/ml	Organ Compound Concentration  Li DMNA 150 µmoles/ml 2.90  Lu DMNA 150 µmoles/ml 3.23  T DMNA 150 µmoles/ml 3.38  - DMNA 150 µmoles/ml 2.89	Organ Compound Concentration  Li DMNA 150 µmoles/ml 2.90 237  Lu DMNA 150 µmoles/ml 3.23 68  T DMNA 150 µmoles/ml 3.38 61  - DMNA 150 µmoles/ml 2.89 42	Organ         Compound Concentration         Total Population Screeneda         Number of Convertants b Ade+ Try+           Li         DMNA         150 μmoles/ml         2.90         237         67           Lu         DMNA         150 μmoles/ml         3.23         68         27           T         DMNA         150 μmoles/ml         3.38         61         11           -         DMNA         150 μmoles/ml         2.89         42         13	Strain D4           Organ Compound Concentration         Total Population Screeneda Ade+ Try+ Ade+ Try+ Ade+         Number of Convertantsb Ade+ Try+ Ade+         Convertantsb Ade+ Try+ Ade+           Li DMNA 150 μmoles/ml         2.90         237         67         81.72           Lu DMNA 150 μmoles/ml         3.23         68         27         21.05           T DMNA 150 μmoles/ml         3.38         61         11         18.05           - DMNA 150 μmoles/ml         2.89         42         13         14.53

= positive control NOTE: PC

SC = solvent and chemical controls DMNA = dimethylnitrosamine

Li = liver Lu = lung T = testes.

= number  $\times 10^5$ a

= number at 10<sup>-1</sup> dilution = contamination present



# ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES:		E			DATE:	7-29-	74	
					Strair	D4		
Organ	Comp	ound	Concentration	Total Population Screened <sup>a</sup>	Numbe Conyer Ade	r of rtants Try	Convertan 10 <sup>5</sup> Surv Ade <sup>+</sup>	its Per vivors Try <sup>+</sup>
Li	FDA	71-72	Н	3.02 (110)	77	15	÷ 25.50	4.97
	FDA	71-72	L	3.71 (135)	92	20	24.80	5.39
Lu	FDA	71-72	Н	3.25 (118)	84	6 ,,	25.85	1.85
	FDA	71-72	L	3.89 (141)	78	6	20.05	1.54
T	FDA	71-72	Н	3.41 (124)	90	13	26.39	3.81
	FDA	71-72	L	4.08 (148)	89	12	21.81	2.94
	Li	Li FDA FDA Lu FDA FDA T FDA	Li FDA 71-72 FDA 71-72 Lu FDA 71-72 FDA 71-72 T FDA 71-72	Li FDA 71-72 H  FDA 71-72 L  Lu FDA 71-72 H  FDA 71-72 L	Organ         Compound         Concentration         Population Screened           Li         FDA         71-72         H         3.02 (110)           FDA         71-72         L         3.71 (135)           Lu         FDA         71-72         H         3.25 (118)           FDA         71-72         L         3.89 (141)           T         FDA         71-72         H         3.41 (124)	Total Number Population Conyer Screened Ade   Li FDA 71-72 H 3.02 (110) 77  FDA 71-72 L 3.71 (135) 92  Lu FDA 71-72 H 3.25 (118) 84  FDA 71-72 L 3.89 (141) 78  T FDA 71-72 H 3.41 (124) 90	Organ         Compound Concentration         Population Screened         Convertants Ade Try           Li         FDA         71-72         H         3.02 (110)         77         15           FDA         71-72         L         3.71 (135)         92         20           Lu         FDA         71-72         H         3.25 (118)         84         6           FDA         71-72         L         3.89 (141)         78         6           T         FDA         71-72         H         3.41 (124)         90         13	Total Number of Population Convertants of Screened Ade Try Ade Try Ade Ade Try Ade Ade Try Ade

NOTE:

TC = test compound

H = high dose

L = low dose

Li = liver

Lu = lung

T = testes
a = number x 10<sup>5</sup>
b = number at 10<sup>-1</sup> dilution
(c) = contamination present

) = percent survival



# ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

SPEC	IES: R	AT			DATE: 8-5-74							
		•			Strain	D4						
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number Convert Ade			ants Per rvivors Try <sup>+</sup>				
PC	Li	DMNA	150 µmoles/ml	2.44	124	74	50.82	23.77				
	Lu	DMNA	150 μmoles/ml	2.73	73 .	10	26.74	. 3.59				
	T	DMNA	150 μmoles/ml	2.72	85	9 - 4 -	31.40	3.42				
SC	-	DMNA	150 μmoles/ml	3.29	74	13	22.52	3.98				
	-	SALINE	-	2.66	99	10	37.22	3.76				

= positive control NOTE: PC

= solvent and chemical controls SC

DMNA = dimethylnitrosamine

Li = liver

= lung Lu = testes .

a

= number x 10<sup>5</sup> = number at 10<sup>-1</sup> dilution = contamination present



# ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES:		RAT			DATE:	8-5-7	<b>'</b> 4	
				•				
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Convertants F Convertants b 105 Survivor Ade Try Ade Tr		ts Per ivors Try <sup>+</sup>	
TC	Li	FDA 71-72	Н	2.81(106)	107	7	38.08(c)	2.49
•,	•	FDA 71-72	L	3.13(118)	79	21	25.24	6.71
	Lu	FDA 71-72	Н	3.78(142)	107	12	28.31	3.17
		FDA 71-72	L	3.83(144)	125	8 . * *	32.64	2.09
		FDA 71-72	H	3.35(126)	131	10	39.10	2.99
	<del></del>	FDA 71-72	L	3.54(133)	112	14	31.64	3.95

NOTE:

TC = test compound H = high dose

= low dose

Li = liver

Lu = lung .

T = testes
a = number x 10<sup>5</sup>
b = number at 10<sup>-1</sup> dilution

(c) = contamination present

) = percent survival



### ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4: POSITIVE AND SOLVENT CONTROL RESULTS

DATE: 9-20-74 SPECIES: MONKEY

					Strain	n D4	of Convertants Per	
Test	Organ	Compound	Concentration	Total Population Screeneda		er of rtantsb Try <sup>+</sup>		
PC	Li	DMNA	150 μmoles/ml	5.67	73	68	12.87	11.99
	Lu	DMNA	150 μmoles/ml	5.46	25	21	4.58	3.85
	T	DMNA	150 μmoles/ml	4.52	16	21(c)	3.54	4.65
SC	<del>-</del> .	DMNA	150 µmoles/ml	5.45	25	21	4.59	3.85
<b>\</b>	<u>-</u>	SALINE	-	5.46	9	15(c)	1.65	2.75

NOTE: PC

= positive control
= solvent and chemical controls SC

DMNA = dimethylnitrosamine

= liver Li

= lung Lu = testes,

= number x 10<sup>5</sup> = number at 10<sup>-1</sup> dilution = contamination present



# ACTIVATION SUSPENSION TESTS WITH SACCHAROMYCES INDICATOR STRAIN D4

SPEC	IES: N	MONKEY			DATE:	J 40 7 !			
					Strain	D4	· · · · · · · · · · · · · · · · · · ·		
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Numbe Conyer Ade			ants Per irvivors Try <sup>†</sup>	
TC	Li	FDA 71-72	Н	3.96 (73)	25	21	6.31	5.30	
		FDA 71-72	L	5.15 (94)	28	22	. 5.44	4.27	
\$	Lu	FDA 71-72	Н	5.98 (109)	16	30	2.68	5.02	
-		FDA 71-72	L	4.89 (90)	33(c)	15 ,.	6.75	3.07	
		FDA 71-72	H H	5.76 (105)	28	26	4.86	4.51	
		FDA 71-72	L L	5.60 (103)	29	0	5.18	-	

NOTE:

TC = test compound

H = high dose

L = low dose

Li = liver

Lu = lung

T = testes

a = number x 10<sup>5</sup> b = number at 10<sup>-1</sup> dilution

(c)= contamination present

( )= percent survival



## XII. SUMMARY OF TEST RESULTS AND INTERFRETATION

Compound FDA 71-72, glucono-delta-lactone, was evaluated for genetic activity in a series of in vitro assays. The following results were obtained.

- A. Salmonella Test Results
- Plate tests

A concentration of 0.5% (w/v) FDA 71-72 was not mutagenic for any of the tester organisms.

2. Non-activation suspension tests

All tests with FDA 71-72 were negative.

3. Activation suspension tests

All tests of FDA 71-72 with mouse and monkey organ homogenates were negative. The initial tests with rat organ homogenates produced apparant increases in three tests with strain TA-1535 and one test with TA-1538. Repeat runs on all suspected tests indicated that the originally observed increases were probably aberrant and did not represent mutagenicity by FDA 71-72.

- B. Saccharomyces Test Results
- 1. Non-activation suspension tests

All tests with FDA 71-72 were negative.

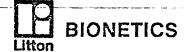
2. Activation suspension tests

All tests with FDA 71-72 were negative. A single data point is absent from the monkey activation tests at the low dose level with testes enzymes. This probably resulted from defective yeast minimal medium. The point was not believed to be of critical importance and was not retested.

## C. <u>Additional Comments</u>

It can be seen that the spontaneous reversion frequencies for the indicator strains are quite variable. This was expected and has been reported in the literature for both the Salmonella and yeast strains. Although cultures with quite low spontaneous frequencies can be selected, we feel that ranges of 1-5 x  $10^{-8}$  for TA-1535 and 5-10 x  $10^{8}$  for TA-1537 and TA-1538 are the mode.

The survival ranges, although not recorded, are also quite variable ranging from over 100% to less than 50% of the control values. It is our feeling



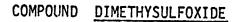
that survival values are dependent on many factors and not only the toxicity of the test compound as determined in the initial survival curves.

Submitted by:

David Brusick, Ph.D. Director, Department of Genetics

## APPENDIX

SUMMARY OF TESTS EVALUATING DMSO FOR GENETIC ACTIVITY IN SALMONELLA AND SACCHAROMYCES



_		
Α.	Suspension	Tacte
/1•	Suspension	16272

	Activat	<u>ion</u>	Salmonella Reversion Frequencies (x 10 <sup>-8</sup> )		Saccharomyce Frequenci	es D4 Conversion les (x 10 <sup>-5</sup> )
Test	Speciesa	0rgan <sup>b</sup>	TA-1535	TA-1538	Ade+	Try <sup>+</sup>
Non-activation	÷	<b>st</b>				
Control (-C) High Dose <sup>C</sup> Low Dose <sup>d</sup>	w: ₩ . ≯ 	<u>.</u> _ € ;	0.74 1.91 0.53	0.88 1.05 1.37	32.51 28.32 40.73	4.34 2.95 0.49
<u>Activation</u>					•	
Control (+C) Control (-C)	- -	- 2	1.80 1.43	0.36 1.04	38.27 37.12	2.47 2.64
High Dose <sup>C</sup>	M M M	Li Lu T 2	0.34 0.59 0.62	1.07 0.58 0.30	. 47.77 36.29 34.35	2.75 1.39 1.94
Lose Dose <sup>d</sup>	M M M	Li Lu T	0.43 0.11	0.87 3.14 0.39	34.02 42.30 45.95	1.18 1.40 2.32

Note: (-C) = solvent control and (+C) = test chemical control without homogenate

a M = mouse Mo = monkey

R = rat

b Li = liver Lu = lung T = testes

Bacteria = 3% Yeast = 5%

Bacteria = 1.5% Yeast = 2.5%

# COMPOUND DIMETHYSULFOXIDE

# B. <u>Plate Tests</u>

	<u>Activation</u>	<u>Sa1</u>	monella Respons	es
Test	Species <sup>a</sup> Organ <sup>b</sup>	TA-1535	TA-1537	TA-1538
Non-activation **	•	·		14-1556
Control (-C) Test compound (3%)	* * *	- -	• • • • • • • • • • • • • • • • • • •	- -
Activation				•
Control (+C) Control (-C)	- - - :	• (		
Test compound (3%)	M Li -M Lu M T	<u>-</u>	·	
	R : Li R Lu R T		<u>-</u> -	. • • • • • • • • • • • • • • • • • • •
	Mo Li Mo Lu Mo - T	- -	- -	- -
Note: (-C) = solvent con	ntrol and (+C) = chemical co	ntrol without homoge	 enate	en en e <del>l</del> Granda de la companya
a M = mouse Mo = monkey R = rat	b Li = 1	iver ung		-